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None

14. ABSTRACT

The purpose of this project is to develop objective peripheral biomarkers for Gulf War Illness (GWI). We plan to determine circulating autoantibodies to ten proteins associated with the central nervous system in sera and plasma from a group of 250 Gulf War veterans with Gulf War Illness (GWI) and from 200 controls (100 healthy GW controls, 100 disease controls). Preliminary results using Western blot assay showed increased levels of autoantibodies in GWI cases compared to symptomatic controls in the following descending order: CaMKII > GFAP > Tau > Tubulin > MAG > MAP-2 > MBP > NFP > S100B ranging from 2-8 fold higher. These results confirm the continuing presence of autoantibodies against neuronal /gliosis in these veterans and are in agreement with recent reports indicating that 25 years after the war, the health of veterans with GWI is not improving and may be getting worse. Such blood-based autoantibodies may prove useful as biomarkers of GWI, upon validation of the findings using larger cohorts.

15. SUBJECT TERMS
Nothing listed

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Table of Contents

1. Introduction

The Subject: Assaying of autoantibodies against neuronal and glial proteins in veterans with Gulf War Illness (GWI); **PURPOSE:** Development of peripheral biomarkers for GWI; **Scope of the Research:** Serum and plasma from 250 Gulf War veterans with GWI and 200 controls (100 healthy GW veterans; 50 chronic fatigue syndrome (CFS) non-veterans) will undergo the study.

2. Key Words

Gulf War Illness, MAP-2, tubulin, NFP, tau, MBP, MAG, CaMKII, GFAP, S100B), Western Blot, Elisa, chronic fatigue syndrome, irritable bowel syndrome

3. Accomplishments

- What were the major goals of the project?
    - The major goals of the project as stated in the approved SOW are listed in the table below. Milestones/target dates for important activities or phases of theses dates and actual completion dates are listed in the table below.
- What was accomplished under these goals?
    - The table lists the proposed goals and dates to accomplish and the actual dates that these goals were accomplished during the first 12-month period

Tasks	**Timeline**	
Task 1: Obtain Regulatory Reviews and Approvals	**Planned Months**	**Actual Months**
1a. Obtain necessary IRB approvals or Exempt status	1-3	1-4
1b. Obtain DOD Human Research Protections Office (HRPO) approvals or Exempt Status	1-3	1-4
Milestone(s) Achieved: Regulatory reviews completed and final approval obtained for study	1-3	1-4
Task 2: Obtain Stored Blood Serum and CSF samples from 3 biorepositories for analysis.	Months	
Proposed 2a: 100 GWI and 50 IBS **serum** samples shipped from Site 5 to Duke and NIH for analysis.	4-6	
Actual 2 a: *100 GWI and 50 IBS* ***plasma*** *samples shipped from Site 5 to Duke and NIH for analysis* ***Actual 2a****: Adapting western blot for plasma samples* ***Actual 2a****: Beginning analysis of all plasma samples from 100 GWI and 50 controls.*		6-8 10-11 10-12
Proposed 2b: 50 GWI and 50 healthy GW veteran controls and 50 CFS control **serum** samples shipped from site 4 to Duke and NIH for analysis. ***Actual 2b****: Plasma samples received from NOVA University:* *1. 50 CFS samples* *2. 26 healthy GW control samples* *3. 68 GWI samples (from Boston GWIC)*	4-9	12

*2c: 100 GWI and 50 healthy GW veteran control **serum** samples shipped from site 1 to Duke and NIH for analysis.	10-22	
Proposed *2d: 25 GWI and 25 healthy GW veteran control CSF samples shipped from site 1 to NIH for analysis	22-24	
Milestone(s) Achieved: Site 1, 4 and 5 serum and CSF data collected and set up for laboratory assays (ELISA, western blot). Autoantibody data shipped to analyzing labs from 250 GWI veterans and 200 controls (100 healthy and 100 diseased controls) blood serum samples and 50 CSF (25 GWI, 25 control) samples.	3-24	Samples were shipped to Duke lab for analysis from three sites including 294 samples (168 GWV; 50 IBS; 50 CFS)
Task 3: Perform Serum Assays	Months	
3a: Perform western blot analyses for autoantibodies to CNS proteins in GWI cases and control samples.	4-24	
3b: Perform ELISA analyses for Neurofascin 155 CNS marker in serum samples from GWI cases and controls.	3-24	
Milestone(s) Achieved: Autoantibodies for CNS proteins of myelinogenesis, astrogliogenesis and neurogenesis data analyzed from three biorepository sites.	9-24	
Task 4: Perform CSF Assays	Months	
4a: Perform ELISA assays of 50 CSF samples for neurofascin 155 biomarker.	22-24	
4b: Merge CSF outcome data with clinical neuroimaging, TBI and exposure data.	24-27	
Milestone(s) Achieved: Antibody for neurofascin 155 marker data analyzed and merged with clinical outcome data from GWIC biorepository site.	24-27	
Task 5: Merge Data and Perform Interim Data analyses	Months	
5a: Merge clinical dataset data from sites 1, 4, 5 case/control status and demographics with results from laboratory analyses performed at NIH and Duke.	10-24	
5b: Data analysis of interim ELISA and western blot results of autoantibodies in GWI cases and controls (healthy and diseased groups) with merged clinical datasets.	18-24	

5c: Discussion of results and preparation of abstracts for meeting presentations and initial manuscript for publication.	18-24	
5d. Annual reports of progress will be written.	12-24	
Milestone(s) Achieved: Preliminary analysis of results and presentation of initial results at scientific meetings and potential publication. Possible biomarker selection for GWI and recommendations for treatment development.	18-24	
Task 6: Perform Final Data analyses and Prepare Manuscripts for Publication	Months	
6a: Merge clinical datasets for neuroimaging, blood and genetic biomarkers, brain injury and exposure history with GWI cases and controls.	25-30	
6b: Perform Data analysis comparing ELISA and western blot autoantibodies outcomes in GWI cases and controls with merged clinical datasets for neuroimaging, blood and genetic biomarkers, brain injury and exposure history.	25-30	
6c: Discuss results of data analyses and prepare abstracts for DOD and other scientific meetings.	25-32	
6d: Preparation of manuscripts • Diagnostic CNS Autoantibody Biomarkers of GWI • Biomarkers of OP pesticide and nerve agent exposures in GW veterans • Biomarkers of prior brain injury in GW veterans	25-36	
6e: Final report of progress will be written.	35-36	
Milestone(s) Achieved: Analysis of all study results, presentation of results at scientific meetings, submitted publication and final report of progress. Possible diagnostic biomarker selection for GWI, brain injury and deployment-related exposures and potential recommendation for treatment development.	35-36	

*** Serum and CSF samples will be collected as part of the ongoing Boston GWI consortium study and will be sent to NIH and Duke study sites as the samples are added to the GWIC biorepository. These samples will all be collected by month 24 of the current study.**

- **What was accomplished under these goals?**
  - *Major activities:* **Sending blood samples from nearly 300 GWV and symptomatic controls from three study sites to Duke Laboratory for analysis.** It was determined during early discussions with other co-investigators that more plasma than serum was available at the study sites so a large aim of this period was to adapt the Western blot assay of autoantibodies from serum samples to plasma samples so that both could be used in this study and importantly in other validation studies and clinical assessments in the future.

- *Specific objectives:* We established Western blot analysis for autoantibodies against neural proteins in plasma samples from GWI subjects and from controls similar to our original method in serum samples from a small pilot sample.
- *Significant results:* These results now allow the running of autoantibody assays not only in serum but also in plasma. This is a considerable advantage because many specimens from GW veterans are prepared as plasma not serum and other stored sample studies could now be used for this purpose.
- *Other achievements.* Currently, we are continuing to adapt our autoantibodies assays against neural proteins to ELISA assays. This will give another dimension in assaying large number of samples to these biomarkers.

a. Materials and Methods

Materials

The sources of standard proteins were the same as stated before (Abou-Donia et al., 2013).

b. Case and control Samples

Serum samples from 10 GWI cases with GWI and 5 controls were tested in this experiment.

c. Western Blot Assay

To screen for the presence of autoantibodies against a battery of proteins in plasma samples, we applied a Western blot approach as previously reported (Abou-Donia et al., 2013). Each serum sample was analyzed in triplicate. Each protein was loaded as 10 ng/lane except for IgG that was loaded as100 ng/lane. Proteins were denatured and electrophoresed in SDS-PAGE (4% to 20% gradient) purchased from Invitrogen (Carlsbad, CA). One gel was used for each serum sample. The proteins were transferred into polyvinylidene fluoride (PVDF) membranes (Amersham Pharmacia Biotech Piscataway, New Jersey). Nonspecific binding sites were blocked with Tris-buffered Saline-Tween (TBST) (40 mM Tris [pH 7.6], 300 mM NaCl, and 0.1% Tween 20) containing 5% non-fat dry milk for 1 h at 22°C. Membranes were incubated with plasma samples at 1:100 dilutions in TBST with 3% non-fat dry milk overnight at 4°C. After five washes in TBST, the membranes were incubated in a 1:2000 dilution of horseradish peroxidase-conjugated goat anti-human IgG (Amersham Pharmacia Biotech (Piscataway, New Jersey). The dot blots were probed with anti- human IgG (H+L) HRP conjugate antibody (Cat. No. 31410, Thermo Fisher Scientific Inc., Pittsburgh, PA, USA) for one hour at RT, incubated with ECL reagent (Cat. No. 34096). The membranes were developed by enhanced chemiluminescence using the manufacturer's (Amersham Pharmacia Biotech) protocol and a Typhoon 8600 variable mode imager. The signal intensity was quantified using Bio-Rad image analysis software (Hercules, California). All tests were performed with the investigators blinded to participant diagnosis.

Results

a. Our preliminary results analyzing the current plasma samples are consistent and confirm the results of our preliminary studies using serum samples.
b. ELISA analysis of the same samples are being analyzing and are showing promising results for future use.

- What opportunities for training and professional development has the project provided?
  Professional development. The results of studies that were generated in this project were presented in a national meeting and an international meeting:
  1. The Annual Meeting of the Society of Toxicology, March 2016, New Orleans: A poster of the results was presented.
  2. The International Neuropsychological Society (INS) annual mid-summer meeting, July 2016, London UK; an oral presentation was given as part of an invited symposium on Gulf War Illness.

- How were the results disseminated to communities of interest?
  - Our results were discussed with many interested scientists during both the SOT and INS meetings.
  - A symposium on the Gulf war Illness took place in London during the International Neuropsychological Society midSociety mid-year meeting last July, during which results from this project and several other related projects were presented and stirred intense interests.
- What do you plan to do during the next reporting period to accomplish the goals?
  - We plan to attend appropriate National and International meetings to present our results from this project.
  - |Annual Meeting of the Society of Toxicology, March 2017, Baltimore, Maryland. An abstract has been submitted for a poster presentation.
  - We submitted and revised a manuscript of the pilot study to the Journal Neurotoxicology and Teratology and are awaiting the next reviewer comments. We hope that this manuscript will be accepted during the next reporting period.
  - We plan to submit other manuscript of preliminary results from the case-control and exposed vs non-exposed groups during the next reporting period as well.

4. Impact
- What was the impact on the development of the principal discipline(s) of the project?
  - Our studies have focused on the development of sensitive, specific, reproducible, and non-invasive blood biomarkers of GWI. Identifying objective biomarkers of GWI helps the veteran and the treating clinicians who must now rely on self-report of symptoms as the primary diagnostic marker. The advantage of a blood-based biomarker is that it can diagnose GWI with greater accuracy and with only a few drops of blood. Our initiative to validate both serum and plasma for these potentially diagnostic autoantibodies will make diagnosing GWI and validating it with other stored blood samples from GWI even easier because it won't be limited by just one type of blood product.
  - The results of the study can be applied immediately to treatment development strategies for the veterans of the Gulf War. Based on the CNS autoantibodies we ultimately find, this will provide the opportunity to develop drugs that treat neuronal injury in those specific pathways (neuronal, glial etc); such treatment could be directly applicable to Gulf War veterans in the short-term.

- What was the impact on other disciplines?
  - A major advantage of our peripheral marker is that it is specific for neural injury irrespective of the cause, thus it can be applied to diagnose or confirm diagnosis of other neurodegenerative diseases such as Alzheimer's disease and Parkinson's disease. However, the *pattern* of the specific markers elevated can be different with different disease states and thus can also be useful diagnostically once validated in different disorders.
  - Blood-based biomarkers of GWI provide an effective way to enhance its management

- It can be used as a diagnostic and prognostic tool with the ability to provide information about rate of disease progression.
- It would help in identification of novel and effective treatments for multiple disorders and environmental exposure groups (i.e. pesticides, nerve agents).
- It could be used for monitoring therapeutic efficacy for the benefit of patients and caretakers.
- It could be used to follow-up treatment plans of the patient
- It could provide a cost-savings potential for recruitment into clinical trials.

- What was the impact on technology transfer?
  - The biomarker that we developed is costis cost effective, available, and does not need expensive technicians that can be used to diagnose neural injury without the need for expensive equipment.

- What was the impact on society beyond science and technology?
  - Our peripheral biomarker should improve the quality of life for the veterans of the GW who have GW illness because:
    - Upon their from thrthe GW theater in 1991, their subjectivrsubjective complaints could not be diagnosed and they were to;adtold that their complaints were "all in their heads". Our biomarker should confirm theit brain injury that is consistent with their complaints. Such consequence should give them a peace of mind.

    Our biomarker should lead to studies to develop treatment of brain injury that may lead to improvrmrntimprovement of their clinical condition.

5. The hallmark of Gulfof Gulf War Illness (GWI) is neuroinflammation, neural cell death in specific regions of the brain and possible progressive neurodegeneration. A challenging aspect of GWI is that it has been difficultbeen difficult to diagnose with objective biomarkers because organophosphate pesticides and nerve agents do not stay in the body and CNS the same way that other exposures do (i.e. agent orange, depleted uranium, lead, mercury). Therefore, researchers have had to develop markers of damage from these chronic exposures rather than markers of the exposure or their bi-products. If successful, this work will impact neurotoxicant exposed individuals including agricultural workers, pesticide applicators and nerve gas exposed groups by providing objective inexpensive markers of chronic damage related these exposures that can be conducted virtually anywhere that a simple blood draw can be obtained and analyzed. Other current diagnostic practices including neuroimaging techniques, behavioral history assessments, and neuropsychiatric tests have drawbacks of not always being practical or available in other parts of the world but a simple blood test could provide objective diagnostic markers in the most cost-effective way. Changes/Problems

- Changes for approach and reasons for change
  - Changes: serum to plasma samples

    Our original studies in determining autoantibodies in blood used serum samples from GWI cases and symptomatic controls. However, it was determined that our co-investigators had more plasma available than serum. Therefore, we carried out experiments to establish the validity of our assay using plasma, as stated above under Accomplishments, which showed that the results from plasma samples were identical to those of serum samples. This was a big accomplishment that either plasma or serum can be used for these analyses because all other major studies of GWI with either serum or plasma samples could potentially validate our findings with their own samples.

  - Problems: ELISA

- We originally requested an equipment "Wes" to facilitate and speed up western plot assay. As soon as our grant was funded, we contacted the company that gave the quote for the equipment, to demonstrate for us how it works. The results were a disappointment:
  - The company's team that carried out the demonstration, could never get a good result.
  - We were informed, for the first time, that the machine uses "kits" for the assay, each kit costs $2,000. Because each serum or plasma sample would require a kit, the cost of the study to assay hundreds of samples, would far exceed our budget.
  - Instead, we decided to develop an ELISA assay for the autoantibodies of all neural proteins. The results of this study are very promising and we plan to use both Western blot and ELISA assays in our studies.
  - The cost of the ELISA system is comparable to the cost of the "Wes" machine, and we will request from our Project Officer the permission to order ELISA equipment instead.

- Actual or anticipated problems or delays and actions or plans to resolve them techniques
  - None is actual or anticipated
- Changes that had significant impact on expenditures
  - None
- Significant changes in use or care of human subjects, vertebrate animals, biohazards, and/or select agents:
  - Significant changes in use or care of human subjects: None
  - Significant changes in use or care of vertebrate animals: None
  - Significant changes in use of biohazards, and/or select agents: None

6. Products

- **Publications, conference papers, and presentations**
  - **Journal Publications**

A manuscript was prepared and submitted for publication to a journal in June. A revised version was submitted in September, and we are waiting for the editorial decision. The manuscript title is: "Screening for Novel Central Nervous System Biomarkers in Veterans with Gulf War Illness"

Mohamed B. Abou-Donia[1], Lisa A. Conboy[2], Efi Kokkotou[3], Eric Jacobson[4,] Eman M. Elmasry[5], Passent Elkafrawy[6] , Megan Neely[7], Cameron R. 'Dale' Bass[8] and Kimberly Sullivan [9]

[1] Department of Pharmacology and Cancer Biology, Duke University Medical Center, Durham, North Carolina, [2]Beth Israel Deaconess Medical Center, Harvard Medical School, Boston, MA, [3] Division of Gastroenterology, Beth Israel Deaconess Medical Center, Harvard Medical School, Boston, MA [4]Department of Global Health and Social Development, Harvard Medical School, Department of Microbiology, Zagazig University, Zagazig, Egypt,[5,] Department of Math and Computer Science, Menoufia University, Shebin ElKom, Egypt[6], Department of Biostatistics & Bioinformatics, Duke University Medical Center[7], Biomedical Engineering Department, Duke University[8], Department of Environmental Health, Boston University School of Public Health, Boston, MA[9]

  - **Books or other non-periodicals, one-time publications**
    - **None**
  - **Other publications, conference papers, and presentations**
    - A poster was presented during the annual Society of Toxicology Meeting at New Orleans entitled:

“ “. See Appendix 1.

- An oral presentation was given in a Symposium on Gulf War Illness during the International Neuropsychological Society (INS) annual mid-year meeting in July, 2016 entitled:
  “A Pilot Study of Novel Brain Neurodegenerative Biomarkers in Veterans with Gulf War Illness: M. B. Abou-Donia[1], K. Sullivan [2], L. A. Conboy[3], E. Kokkotou[4,] and E. M, El-Masry[5] [1]Duke University Medical Center, Durham, NC, [2] Boston University School of Public Health, Boston, MA, [3]Harvard Medical School, Boston, MA, [4] Harvard Medical School. Boston, MA ; [5] Zagazig University, Zagazig, Egypt”

- **Other products**

- **Website(s) or other Internet site(s)**
  - **None**

- **Technologies or techniques**
  - **None**

- **Other products**
  - **None**

7. Participants & other Participating Organizations

Site 1: Boston University School of Public Health
715 Albany Street, T4W
Boston, MA 02118
Initiating PI: Dr. Kimberly Sullivan
Co-I: Dr. Joseph Massaro
Co-I: Dr. Maxine Krengel
Tasks 1-6

Site 2: Duke University Medical Center
Durham, North Carolina 27710
Partnering PI: Dr. Mohamed Abou Donia
Co-I: Dr. Cameron R. ‘Dale” Bass
Tasks 2,3,5,6

Blood Serum and CSF Biorepository Sites

Site 3: National Institutes of Health, NICHD
Bldg. 35, Room 2A211, MSC 3713
35 Lincoln Drive
Bethesda, MD 20892

Site PI: Dr. R. Douglas Fields
Co-I: Dr. Dipankar Dutta
Tasks 2-6

Site 4: NOVA SoutheasternNOVA Southea
Ft. Lauderdale, FL
Co-I: Dr. Nancy Klimas
Tasks 1,2, 5, 6

Site 5: Beth Israel Deaconess Medical Ctr.
Boston, MA 02118
Consultant: Dr. Efi Kokkotou
Consultant: Dr. Lisa Conboy
Tasks 1,2, 5,6

Study Sites Responsibilities

Site 1: Dr. Sullivan and her BUSPH team will be responsible for providing the serum blood and cerebrospinal fluid samples from GWIC study participants who have agreed to share their specimens with the GWIC biorepository to be used in future studies including the proposed study. Specifically, she will oversee the recruitment and blood draws/lumbar punctures of study participants from the GWIC study and the processing of serum and CSF samples that will be shared for the proposed study. Dr. Sullivan will also assist with the experimental design, data analysis and interpretation and presentation of study results in collaboration with Dr. Abou Donia and the other study investigators. **Tasks 1-6**

Site 2: Dr. Abou-Donia will be responsible for receiving the serum and plasma samples from all sites and performing autoantibody analyses using western blot/elisa analyses for 450 serum samples (250 GWI, 200 controls). He will also assist with the experimental design, interpretation of data, report and manuscript writing and presentation of results at scientific meetings. **Tasks 2, 3, 5, 6**

Site 3: Dr. Fields will be responsible for receiving the serum and CSF samples from all study sites and performing ELISA assays for 450 serum/plasma samples and 50 CSF samples. He will assist with the experimental design, interpretation of data, report and manuscript writing and presentation of results at scientific meetings. **Tasks 2-6**

Sites 4 and 5: Drs. Klimas, Conboy and Kokkoutu will provide serum samples from their respective biorepositories for study analyses, will assist with interpretation of data, report and manuscript writing.

8. Special Reporting Requirements 10
   None

9. Appendices

   Appendix 1. An Abstract that was presented as a poster during the society of toxicology annual meeting in March 2016, in New Orleans Louisiana.

   Appendix 2. An Abstract that was presented during the International Neuropsychological Society meeting in London, UK

Appendix 1

A. Annual Society of Toxicology Meeting, New Orleans, LA, March, 2016

A Pilot Study of Novel Brain Neurodegenerative Biomarkers in Veterans with Gulf War Illness. MB. Abou-Donia[1], K Sullivan [2], L A. Conboy[3], and E Kokkotou[4]

[1]Duke University Medical Center, Durham, NC, [2] University of Boston, Boston, MA, [3]Harvard Medical School, Boston, MA, [4]Harvard Medical School. Boston, MA

Upon their return from the 1990-1991 Gulf War (GW), hundreds of thousands of American military personnel complained of symptoms with unknown etiology, known as the Gulf War IllnessWar Illness (GWI). The hallmark of GWI is neural degeneration that is consistent with symptoms related to nervous system injury and confirmed by experimental studies. A major problem in identifying veterans with GWI is the difficulty in its diagnosis. This report presents the results of a pilot, investigative and descriptive study of assays performed to detect circulating autoantibodies to a panel of nine proteins associated with the nervous system in sera of a group of 20 veterans of the Gulf War who reported having symptoms of Gulf War Illness (GWI) and 10 symptomatic controls who did not have GWI. Various types of proteins present in axons, dendrites and myelin sheath that are affected by neuronal degeneration were used. In sera samples from the GWI subjects and symptomatic non-veteran controls using Western blotting, immunoglobin IgGs were measured against: neurofilament triplet proteins (NFP), tubulin, microtubule associated tau proteins (tau), microtubule associated protein-2 (MAP-2), myelin basic protein (MBP), myelin associated glycoprotein (MAG), glial fibrillary acidic protein (GFAP), calcium-calmodulin kinase -2 (CAM-2) and glial S100B protein. Also, α-synuclein, a marker for Parkinson' disease was included. The results show significantly elevated levels of circulating Ig-G-class autoantibodies in the veterans with GWI, compared to controls. This preliminary study demonstrates a relationship between clinical condition, and the level of serum autoantibodies to nervous system-specific proteins. These results showing the development of neuronal injury and gliosis in the subjects are consistent with recent reports indicating 20 years after the Gulf War, the health of the- veterans who developed GWI is getting worse. It is concluded that that these serum circulating autoantibodies may be used as biomarkers for

confirming GWI upon further validation. (Supported in part by DOD Contracts No. W81XWH-15-1-0641 and W81XWH-15-1-0640).

Abstract 2.

B. International Neuropsychological Society (INS) annual mid-year meeting, July 2016.

London Abstract: A Pilot Study of Novel Brain Neurodegenerative Biomarkers in Veterans with Gulf War Illness: M. B. Abou-Donia[1], K. Sullivan [2], L. A. Conboy[3], E. Kokkotou[4,] and E. M, El-Masry[5] Duke University Medical Center, Durham, NC, [2] Boston University School of Public Health, Boston, MA, [3]Harvard Medical School, Boston, MA, [4] Harvard Medical School. Boston, MA ; [5] Zagazig University, Zagazig, Egypt

Objective: To determine circulating autoantibodies in ten proteins associated with nervous system in sera of a group of 20 veterans of the Gulf War who reported having symptoms of and 10 symptomatic non-veteran controls with lower back pain. 2. Participants and Methods: Subjects and controls were obtained from the Beth Israel Deaconess Medical Center and Harvard Medical School bio-repository. Control serum samples came from a separate study of non-veteran patients with chronic lower back pain who served as 'symptomatic' controls from one of the authors (EK). The proteins were separated using Western blot assay. 3. Results: Mean levels of autoantibodies in the subjects compared to controls were in descending order: CaMKII (9.27) > GFAP (6.60) > Tau (4.83) > Tubulin (4.41) > MAG (3.60) > MAP-2 (2.53) > MBP (2.50) > NFP (2.45) > S100B (1.03). 4. Conclusions: This study demonstrates a relationship between clinical condition, and the level of serum autoantibodies to nervous system-specific proteins. It is concluded that that these serum circulating autoantibodies may be used as biomarkers for confirming GWI when validated in larger samples. (Supported in part by DOD Contracts No. W81XWH-15-1-0641 and W81XWH-15-1-0640).